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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OCT 19 1999

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
INTERNATIONAL ACTIVITIES

Memorandum:

Subject: Human study Reviewed on Acute intake of Methomyl

From: Henry W. Spencer, Ph.D. 10/14/99
SAB/ HED/OPP (H7509C)

TO: Tom Myers, PM Team Reviewer
Reregistration Branch/ SRRD

This memorandum transmits the finished review of an oral human testing study for methomyl. This study was submitted as a 6 (a) (2) piece of information as MRID 44721401.

EPA Reviewer: Henry Spencer, Ph.D. Date 8/5/99
Review, SAB __ (7509C)
EPA Secondary Reviewer: Robert Zendzian, Date 8/2/99
Review Section SAB Toxicology Branch — (7509C)

DATA EVALUATION RECORD

013802

STUDY TYPE: Acute, single oral capsule dose toxicity in human males (No guideline numbers or requirements apply for this study)

DP BARCODE: D253743
P.C. CODE: 090301

SUBMISSION CODE: -
TOX. CHEM. NO.: -

TEST MATERIAL (PURITY): *[Ethanimidothioic acid, N-[[(methylamino) carbonly]oxy]-methyl ester (Methomyl) approximately 89% ai.*

SYNONYMS: *Lannate SP, DPX-X1179*

CITATION: P. McFarlane, J.B. Sanderson, S. Freestone (30 November,1998) A Randomised Double Blind Ascending Oral Dose Study with Methomyl to Establish a No Adverse Effect Level. Inveresk Clinical Research, Edinburgh, Scotland. Laboratory report HLO-1998-00969, 30 November 1998. MRID *44721401*. Unpublished

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EXECUTIVE SUMMARY:

In a single dose acute toxicity study (MRID #*44721401*), Methomyl, *(%a.i.)]* was administered to *male humans in groups of 5*/dose *by capsule* at dose levels of 0.1, 0.2, 0.3 mg/kg/day). Four persons received only empty capsules as a control group.

There is no overt toxicity with the exception of a single person who showed significant red bloodcell cholinesterase inhibition, increased salivary output and a headache which was assumed to be the result of the chemical effect on the blood vessels. There were no compound related effects in mortality, clinical signs, body weight, food consumption, hematology, clinical chemistry, organ weights, or gross and histologic pathology in anyother person excepting the cholinesterase effects in red blood cell, plasma and salivary output. **The LOEL is 0.1 mg/kg, based on red blood cell cholinesterase inhibition and increased salivation in a limited number of individuals. A NOEL is not established for this study.**

This acute, single dose toxicity study is classified *supplementary*

and does not satisfy a guideline requirement for an acute oral study (82-1). It is not upgradable.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: *Methomyl, Lannate Sp*
   Description *Formulation[of white solid material]*: The study author states that the test substance ▒appeared▒ to be stable under the conditions of the study.
   Lot/Batch #: DPX-X1179-512
   Purity: approximately 89% ai.with approximately 11% inerts of mostly hydrated silica
   Stability of compound: Untested
   CAS #: 16752-77-5
   [Structure]

2. Vehicle control: *the material was weighed out as dry solid into a gelatin capsule without additional vehicle.*
   The empty capsule was the vehicle control.

3. Test animals: Species: Human, males

   Strain: mostly Caucasian and only 1 Asian
   Age and weight at study initiation: The mean age for each of the 4 groups tested ranged from 22.6 to 29 years. Ages ranged from 18 to 37 years. Weights ranged from 60.6 Kg to 91.9 Kg.

   Source: Appeared to be from the local populous.

   Housing: The dosing and examinations occurred in the laboratory facility and occurred on a single day excepting for the post examination call back after 5-7 days.

   Diet: *A standard laboratory diet breakfast was taken within a 15 minute period just prior to taking the test material capsule.*

   Water: 5 minutes *After eating, the test dose in capsule was then taken* and followed by 150 ml water.

Environmental conditions: Temperature: NA
Humidity:NA
Air changes:NA
Photoperiod:NA

Acclimation period: 16 hrs prior to dosing.

B. STUDY DESIGN:

1. In life dates - start: 21 November,1997 end: 15 Dec., 1997
2 persons started 21 Nov.,1997 and ended 27 Nov. 1997
6 persons started 28 Nov.,1997 and ended 1 Dec., 1997
5 persons started 2 Dec.,1997 and ended 9 Dec.,1997
6 persons started 15 Dec.,1997 and ended 21 Dec.,1997.
Note: the length of in life dates were 7 days or up to 2 days less.

2. Subject assignment

Individuals were assigned by *computer generated randomization lists* to the test groups in table 1.

TABLE 1: STUDY DESIGN

Test Group	Conc. in dose *(mg/kg)*	Dose to Animal *(units)*	Male	Female
Control	na	0	4	0
Low	na	0.1	5	0
Mid	na	0.2	5	0
High	na	0.3	5	0

3. Diet preparation and analysis was not involved in this study, though the persons on study received a light standard lunch and after 3 hrs postdosing, fluids were provided on request in the study period.

4. Statistics - The study was stated to evaluate the NOAEL of treated groups with respect to a placebo group (control). The statistical methods used in that evaluation were correctly chosen. However the placebo group was limited to only 4 individuals which is not an adequate number to use as an untreated cohort. The analysis which should have been used is that of a paired t-statistic which compares the pre

and post treatment values of the same individual. This later comparison was carried out in this review. Additionally, whether a statistical evaluation with such small numbers in a group could be carried out or not is a moot point. This emanates from the fact that even small numbers of individuals reacting to the treatment should be considered an effect level.

C. METHODS:

1. Observations:

The subjects were observed for signs of toxicity and morbidity throughout the length of the study. The subjects were also examined prior to the start of the study.

2. Body weight

The subjects were weighed in order to determine the doses to be dispensed.

3. Food consumption and compound intake

Food consumption was not a factor concerning compound intake in this study.

4. Ophthalmoscopic examination

Eyes were examined prior to the start of the study and measurements of the reactivity of the pupil to a given intensity of light and recovery at specified time periods after treatment. Measurements were determined as mm of diameter of the pupils.

5. Blood was collected *from indwelling venous catheters in the arms of the test individuals. Base line values were determined 16 hrs before the study as well as 30 minutes before the study started and at 15 minute intervals for the first 2 hours, then at 3,4,6,8,12, and 24 hours after treatment. Fasting was not a requirement for test values excepting for the 16 hr and 30 min times before the test material was received.* The CHECKED (X) parameters were examined.

a. Hematology

x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)
x	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Vital signs-

Systolic and diastolic blood pressure changes were determined while in the supine and erect positions-as well as the body temperature at screening, and on admission day at -16 hr, -30 min., 1 hr., 2 hr., 3 hr., 4 hr., 8 hr., and 24 hr times.

C. Clinical Chemistry

Clinical chemistry values were obtained only at the 30 minute preexposure and the 24 hour post exposure time periods.

	ELECTROLYTES		OTHER
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
x	Magnesium	x	Blood urea nitrogen*
x	Phosphorus*		Total Cholesterol
	Potassium*	x	Globulins
	Sodium*	x	Glucose*
		x	Total bilirubin
	ENZYMES	x	Total serum protein (TP)*
x	Alkaline phosphatase (ALK)		Triglycerides
x	Cholinesterase (ChE)		Serum protein electrophores
x	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
x	Serum alanine amino-transferase (also SGPT)*		
x	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis*

* Not required for subchronic studies

Urine was collected from 24 hr fasted individuals at the -16 hour prestudy screening and at 24 hrs post dosing time points. The CHECKED (X) parameters were examined.

	Appearance	x	Glucose
	Volume	x	Ketones
x	Specific gravity	x	Bilirubin
x	pH	x	Blood
x	Sediment (microscopic)		Nitrate
x	Protein	x	Urobilinogen

7. Sacrifice and Pathology

There was no sacrifice or pathology to be reported in this study on humans.

II. **RESULTS:**

A. Observations :

1. Adverse events- observations were on a continuous basis for 24 hours. Only 1 patient reported a problem which was considered to be possibly related to treatment. The effect was a headache which occurred 1 hr and 45 min. after dosing at the 0.3 mg/kg dose.

2. Toxicity -There was no toxicity reported other than the lowering of RBC and plasma cholinesterase activity and increased salivation (at 0.2 mg/kg and above) as noted when using the statistical method chosen by the testing laboratory. It evaluated the group value of each dose level compared to the control group value. In all cases of cholinesterase evaluations in the study it is noted that the power of this study is quite limited and only provides data that may approach the true significance of the inhibitions noted. Furthermore, the evaluation of plasma cholinesterase inhibition by carbamates when using the Elman method is known to underestimate the true inhibition of the enzyme(s) in the plasma.

When the data were subdivided into more relevant groupings for individual pre and post treatment comparisons (paired comparisons), it was found that effects were significant at lower doses.

However, excessive salivation was also reported.

Again by the statistical methods chosen by the testing laboratory, salivation was excessive at both 0.2 and 0.3 mg/kg treatment levels. This review considers the increased salivation of +133% in even 1 of the 5 individuals at 1 hour to be a significant increase at the lowest level when associated with reduced red cell CHE activity.

RED BLOOD CHOLINESTERASE ACTIVITY (%change) compared to control unit value

Dose Levels

	Control value Units	Time post dosing 1hr: Units	1hr 15min Units
002	9159	9951 (8.6)	8601 (-6.1)
004	12252	8355 (-31.8)	10902 (-11)
012	12222	12318 (0.7)	10953 (-10.4)
019	13365	13854 (3.6)	12744 (-4.7)
0.1 mg/kg		1hr:	1hr 15 min
001	13059		8154 (-37.5)
003	10662		9378 (-12.0)
006	7806		7056 (-9.6)
007	10170	6753 (-31.4)	8136 (-20.0)
008	10734		7074 (-34.1)

0.2 mg/kg

		45 min	1 hr:
015	11022	7773 (-29.4)	8724 (-20.8)
016	11691	7935 (-32.1)	7086 (-39.4)
017	11109	11418 (2.8)	8772 (-21)
018	13608	9357 (-31.2)	9099 (-33.1)
020	13080	10566 (-19.2)	10644 (-18.6)

0.3 mg/kg

		15 min	30 min	1 hr:
005	9963	6780 (-31.9)	7401 (-25.7)	7344 (-26.2)
009	12063	11142 (-7.6)	7455 (-38.2)	9216 (-23.6)
011	10278	7932 (-22.8)	7218 (-29.8)	7863 (-23.5)
013	11388	9072 (-20.3)	7320 (35.7)	6945 (-39)
014	12177	9966 (-18)	7878 (-35.3)	8523 (-30)

( ) % change from individual control value

Data from the red blood cell acetylcholinesterase determinations indicates that as much as 34% to 37% inhibition occurred in several of the individuals at the lowest dose level. The LEL for inhibition of red cell CHE is 0.1 mg/kg.

Salivation values at 1 and 2 hours are compared to the values reported at the -30 min time for the above animals and are presented in the following table.

Dose levels	GRAMS OF SALIVA FORMED in 5 minutes			
Placebo		1 hour	% change	2 hour % change
0.0 mg/kg	-30min.			
002	4.1	3.6	(-12)	2.6 (-36)
004	3.5	3.0	(-14)	3.0 (-14)
012	1.9	1.8	(-5)	1.4 (-26)
019	0.9	0.7	(-22)	1.0 (+11)
0.1 mg/kg				
001	0.8	0.6	(-25)	1.0 (+25)
003	0.5	0.5	(0.0)	0.3 (-40)
006	2.1	4.1	(+95)	3.2 (+52)
007	0.3	0.7	(+133)	0.4 (+33)
008	1.0	0.9	(-10)	1.1 (+10)
0.2 mg/kg				
015	1.2	1.9	(+58)	2.2 (+83)
016	0.2	0.5	(+150)	0.4 (+100)
017	1.1	1.5	(+36)	1.0 (-9)
018	3.0	4.2	(+40)	5.2 (+73)
020	1.2	1.8	(+33)	1.2 (0.0)
0.3 mg/kg				
005	2.1	3.6	(+71)	1.9 (-9)
009	1.8	2.2	(+22)	1.6 (-11)
011	1.7	3.3	(+94)	2.5 (+47)
013	1.1	1.8	(+63)	1.2 (+9)
014	0.3	1.5	(+400)	0.7 (+133)

The lowest dose of 0.1 mg/kg is an LEL based on 1 of 5 individuals at 1 hr and increasing to 2 of 5 at 2 hrs.with significant increase in salivary output.

PLASMA CHOLINESTERASE UNIT ACTIVITY and %CHANGE FROM -30 minute CONTROL VALUES

Dose -30 min		15 min	30 min	45 min	1 hr	1:15 hr
0.0 mg/kg						
002	6502	-	-	-	6130(-5.7)	6073(-6.6)
004	5193	-	-	-	5361(+3.2)	5369(+3.4)
012	5017	-	-	-	4740(-5.5)	4585(-8.6)
019	5643	-	-	-	5917(+4.8)	6036(+7.0)

Dose and patient		TIME IN HOURS AND MINUTES AFTER DOSE				
0.1 mg/kg		:15	:30	:45	1:00	1:15
001	6295	-	-	-	6197(-1.5)	6097(-3.1)
003	6222	-	-	-	5885(-5.4)	5843(-6.1)
006	4395	-	-	-	4011(-8.7)	4076(-7.2)
007	4401	-	-	-	4121(-6.4)	4104(-6.7)
008	5055	-	-	-	5008(-1.0)	5476(+8.3)
0.2 mg/kg		:15	:30	:45	1:00	1:15
015	4568	-	-	3760(-17.7)	4068(-10.9)	-
016	5906	-	-	5370(-9.1)	5291(-10.4)	-
017	5752	-	-	4973(-13.5)	4896(-14.9)	-
018	5378	-	-	4996(-7.1)	5062(-5.9)	-
020	4760	-	-	4786 (+0.5)	4629 (2.7)	-
0.3 mg/kg		:15	:30	:45	1:00	1:15
005	5332	4995(-6.3)	4726(-11.4)	-	4485(-15.9)	-
009	5647	5172(-8.4)	4721(-16.4)	-	4759(-15.7)	-
011	5185	4339(-16.3)	4106(-20.8)	-	4286(-17.3)	-
013	5604	5122(-8.6)	4876(-13)	-	4830(-13.8)	-
014	6556	5951(-9.2)	5390(-17.8)	-	5213(-20.5)	-

The 0.3 mg/kg data indicate that after ingestion of the capsule with methomyl, it started to dissolve as early as 15 minutes as is seen with person 011 with -16.3 percent less than the control value taken just 45 minutes prior. The optimum time at the highest dose (0.3 mg/kg)for plasma cholinesterase effects appears to be between 30 minutes and 1 hour.

Though only a small number of individuals were used in the study, the control group data collected suggests that the plasma cholinesterase activity varied over 1 ½ hours to 2 hours

(starting at -30 minutes to 1hr and 15 minutes after dosing) by only + or - 8%. If this is true, then plasma cholinesterase was reduced only at the higher two doses (0.2 and 0.3 mg/kg). The LEL is 0.2 mg/kg with a NOEL of 0.1 mg/kg for plasma cholinesterase inhibition.

3. Blood pressure, heart rate or temperature changes:

There were a few sporadic changes in the rate when the individuals were in the supine and also erect position. However, the changes were not dose related and several occurred at time periods which would not have been considered to be treatment related. Overall, there were no consistent patterns supporting blood pressure, heart rate or body temperature changes related to exposure from the chemical.

4. Mortality - There was no mortality reported in the study.

B. Body weight and weight gain: *There was no information taken on body weight changes in the study.*

C. Food consumption and compound intake :

1. Food consumption - There were no measurements on food consumption.

2. Compound consumption: There was only a one time dose at the beginning of the study.

3. Food efficiency : Not relevant

D. Ophthalmoscopic examination -

Ophthalmic examination was only completed as part of an evaluation of the reactivity of the pupillary response to a cholinesterase inhibitor. The data did not show a response related to the increasing dosages.

E. Blood work:

1. Hematology - There were no significant changes which were considered to be related to treatment.

2. Clinical Chemistry -

Changes in serum chemistries were minimal and were not dose related nor consistent with time. It is considered that a single oral capsule treatment with methomyl had no

effect on these parameters.

F. Urinalysis - There were no changes which could be considered associated with the treatment compound.

G. Sacrifice and Pathology: All sections concerning possible changes in this study were not relevant

1. Organ weight -

2. Gross pathology -

3. Microscopic pathology -

a) Non-neoplastic - NA

b) Neoplastic - NA

III. DISCUSSION

A.
Following the exposure of the individuals to methomyl by capsule, the only significant changes in the large number of parameters evaluated were those for red blood cell, plasma cholinesterase and the associated increased salivary volume output.

Only 1 person at the highest dose level (0.3 mg/kg) produced an adverse event other than the simultaneous increased salivary output.

Further discussion with individuals in the Agency notes that the relatively lower inhibition of the plasma cholinesterase activity is known to occur and is considered to underestimate the real inhibition of the plasma cholinesterase by carbamates.

Since excessive salivation is one of the signs to accompany this chemical's activity on the red cell enzyme, this reviewer would consider the effect to also be an adverse effect. This effect is also probably one of the most sensitive effects since the trigger is perhaps only one or two synapses away from effecting excessive flow of saliva. One of the important aspects of salivary output is the hydation state of the individual and therein lies the possible reason for the variation in some of the individual output volumes. Data herein does not allow the evalatuation of the internal state of hydration of the test individuals. True, the resulting headache of the individual at 0.3 mg/kg is significant in that it is a painful event-supposedly resulting from dilatation of blood vessels in the brain-to that

person and with a small amount more of the chemical a truly perilous condition could result. Therefore, based on the decreased red cell cholinesterase activity at 0.1 mg/kg and the simultaneous increase in salivary output, the LOAEL is established at 0.1 mg/kg without an NOAEL found in the study. The study does not fulfill any guideline requirements and is strictly a piece of supplementary information. Based on the intent of the study, and the analytical methods used in its evaluation by the registrant, its objective was fulfilled.

B. Study deficiencies

The major deficiency in this study is the small number of individuals in each group. A second deficiency is the fact that this study only utilized males.

The use of small numbers of individuals limits the usefulness of the data for group comparisons and also makes the limited occurrence of any one or two individuals reacting a more significant effect.

A fourth deficiency in this study is the lack of testing to determine which cholinesterase method would be most appropriate for the plasma activity determination. There was no reported standard tested in this study. However, the deficiency for plasma testing would not deter the usefulness of the red cell activity determinations.

The fact that the control red cell cholinesterase value of 1 control individual was -31% at 1 hr post dosing and was not retested while the next sample at 1hr 15 min. was approx. 60% higher at -11% (only 15 mins later) did not raise a question with the study directors only suggests that evaluation of conduct of the study was somewhat lax until large segments of the data were assembled.

A sixth deficiency in this study is the use of capsules in the delivery of the methomyl to the patient. As may be seen from the data in the red cell CHE determinations, the variability of the dissolution of the capsule is apparent. Obviously the capsule had to be completely dissolved at the lowest doses to liberate the total dose to effect the red cell CHE change. With the higher dose of 0.3 mg/kg the capsule only had to be slightly dissolved as seen after 15 min or 30 minutes to provide significant red cell CHE inhibition.

The study would have provided more consistent data if the material had been given as a liguid and not as a capsule on top of additional food to impede its dissolution and absorption in a variable manner as is seen in this study..

If a realistic study was envisioned, and one which would be useable by risk assessors, consultation with persons using that data would have been advised.

The study is only considered as supplementary data and is not upgradable.

An additional table of mean RBC cholinesterase, mean percent change from baseline is appended which shows the percent change from baseline over the entire 24 hr study.

013802

Methomyl. Single dose acute toxicity study in humans (MRID 44721401). comments re review by H Spencer PhD dated 7/17/99

8/2/99

Robert P. Zendzian PhD
Senior Pharmacologist

In conversation with W. Burnam it is recommended that section 7 of the review, Sacrifice and Pathology, be deleted as this was a recovery study and the section is not necessary.

I agree fully with the conclusions of the review. A NOEL was not established for erythrocyte cholinesterase inhibition. A dose-related decrease in mean erythrocyte cholinesterase activity was observed with a LOEL of 0.1 mg/kg, the lowest dose tested. This is clearly shown in Table below, where a maximum decrease in activity is observed at 1:15 hr, 1:15 hr and 0:45hr for doses of 0.1, 0.2 and 0.3 mg/kg respectively.

Table A. Methomyl. RBC cholinesterase,mean percent change from baseline.Data from Table R2.2 of the report.

Time of Sample	Dose			
	placebo	0.1mg/kg	0.2 mg/kg	0.3 mg/kg
-16hr				
-30min				
+15min	5.8	3.1	-1.2	-18.6
+30min	-1.8	-9.2	-12.4	-32.0
+45min	-2.9	-2.4	-20.0	-35.3
+1hr	-4.0	-14.6	-24.7	-27.3
+1hr15min	-4.3	-19.0	-27.6	-26.8
+1hr30min	-0.3	-10.6	-27.9	-23.2
+1hr45min	1.8	-3.7	-22.2	-22.4
+2hr	5.8	-8.9	-16.2	-16.0
+3hr	12.1	-2.1	-1.3	-12.9
+4hr	11.4	5.0	-2.3	-5.0
+6hr	6.2	-5.9	14.8	-2.0
+8hr	-4.4	-8.7	9.0	-0.5
+12hr	-5.7	-15.3	14.4	-5.6
+24hr	-4.9	-3.7	10.0	-1.4